REM sleep in naps differentially relates to memory consolidation in typical preschoolers and children with Down syndrome

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Edited by Terrence J. Sejnowski, Salk Institute for Biological Studies, La Jolla, CA, and approved September 24, 2018 (received for review July 4, 2018)

Sleep is recognized as a physiological state associated with learning, with studies showing that knowledge acquisition improves with naps. Little work has examined sleep-dependent learning in people with developmental disorders, for whom sleep quality is often impaired. We examined the effect of natural, in-home naps on word learning in typical young children and children with Down syndrome (DS). Despite similar immediate memory retention, naps benefitted memory performance in typical children but hindered performance in children with DS, who retained less when tested after a nap, but were more accurate after a wake interval. These effects of napping persisted 24 h later in both groups, even after an intervening overnight period of sleep. During naps in typical children, memory retention for object-label associations correlated positively with percent of time in rapid eye movement (REM) sleep. However, in children with DS, a population with reduced REM, learning was impaired, but only after the nap. This finding shows that a nap can increase memory loss in a subpopulation, highlighting that naps are not universally beneficial. Further, in healthy preschooler's naps, processes in REM sleep may benefit learning.

naps | sleep | memory | development | Down syndrome

In our productivity-driven society, the benefits of sleep for our long-term health and new learning are often ignored. The capacity of a full night's sleep or short naps to improve learning has been observed in healthy adults (1–3), infants, and preschoolers (4–6). While considerable evidence indicates that sleep promotes active memory consolidation (7), theories also suggest that sleep facilitates memory stabilization by reducing the impact of overactive synapses through synaptic downscaling (8). Studies investigating the relationship between napping and memory consolidation have focused primarily on healthy groups, raising the question of whether naps are always beneficial across populations. Indeed, napping's universal utility is a current debate. The potential for cognitive benefits through naps is an open question as the neural processes involved in sleep and memory formation can differ throughout healthy development and in individuals with developmental disorders (9–11).

In this study, we assessed whether children with Down syndrome (DS, trisomy 21), a condition associated with sleep disorders, receive the same benefits from daytime naps as typically developing (TD) children (4, 6). DS is the most common genetic form of intellectual disability (12) that carries a unique cognitive profile characterized by cognitive deficits, language difficulties (13), and hippocampal-dependent memory deficits (14, 15). Despite decades of research characterizing the cognitive profile of DS, the role of disturbed sleep in cognitive deficits is largely unexplored in this population.

What physiological processes allow the sleep experienced while napping to be beneficial? Both rapid eye movement (REM) and non-REM (NREM) stages of sleep contribute to the consolidation of nascent material (7, 16–18). NREM 3 (N3, slow wave) sleep orchestrates hippocampal–neocortical dialogue and information transfer (19, 20). Recent work sheds new light on the importance of REM sleep as well (16, 21, 22), with NREM and REM potentially working in concert to facilitate learning. DS provides an interesting condition to examine the effects of sleep disturbance due to well-replicated REM disruptions in the context of broader sleep impairments, including sleep apnea (23).

We investigated word learning across naps and periods of wakefulness in preschoolers with and without DS. Our aims were: (*i*) to examine how preschoolers with DS learn after naps compared with TD children and (*ii*) to relate sleep parameters to learning outcomes. To measure learning, we assessed retention of new words (auditory labels for novel visual stimuli) across different intervals, with one interval containing a nap. Each child was tested on novel word learning in three counterbalanced, within-subject conditions: (*i*) 5-min delay, (*ii*) wake, and (*iii*) sleep (Fig. 1*A*). In the 5-min delay condition, children received the recognition test 5 min after the training phase, whereas in the wake and sleep conditions, children were tested after 4 h as well as 24 h later to examine long-term retention. Sleep physiology was assessed with nap polysomnography (PSG).

Results

Behavioral analyses were conducted in 25 children with DS [52% female; mean (SD) age = 54.16 (9.49) mo] and 24 TD children [54% female; mean age = 33.38 (5.05) mo]. Groups were

Significance

This paper demonstrates that typical children have enhanced learning of new words across sleep periods (naps) which is linked to the amount of time in rapid eye movement (REM) sleep and shows sleep-dependent learning losses in an atypically developing group of children with REM deficits (e.g., Down syndrome). The work yields both medical and theoretical impacts by (*i*) highlighting a modifiable mechanism of intellectual disability in Down syndrome that has not been described before and (*ii*) emphasizing the important role of REM sleep in children's learning.

Author contributions: G.S., R.L.G., and J.O.E. designed research; G.S. and B.I.D. performed research; M.A. and S.L.C. contributed new reagents/analytic tools; G.S. and J.O.E. analyzed data; and G.S. and J.O.E. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The data reported in this paper have been deposited in Open Science Framework and can be accessed using the following website https://osf.io/tuab9/.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1811488115/-/DCSupplemental.

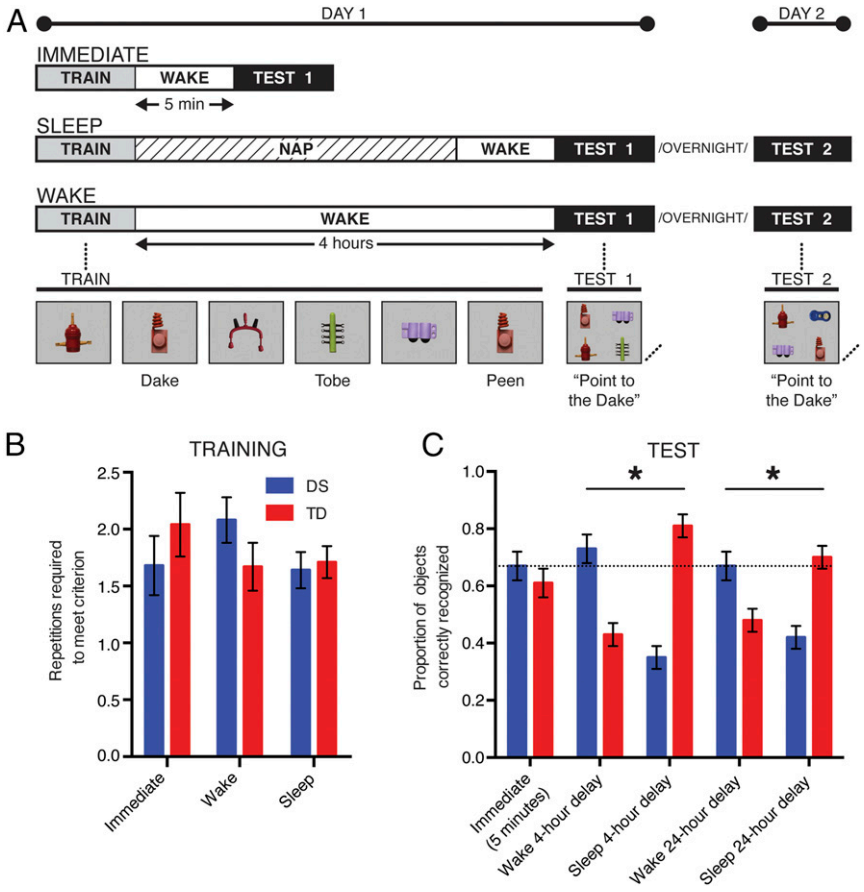

Fig. 1. Protocol design and behavioral results. (*A*) Protocol design and object-label association task. Each child was tested with an object-label pairing task in three counterbalanced, within-subject conditions separated by 1–2 wk: (*i*) immediate, (*ii*) wake, and (*iii*) sleep. Every condition included two phases: training and test. During the training phase, children were exposed to three novel object-label mappings and three novel unlabeled distractors, trained to a set criterion (four out of six correct trials, 66.7%). Both 4- and 24-h tests included six trials. (*B*) Training: Number of repetitions required to meet criterion in children with DS (in blue) and TD toddlers (in red). (*C*) Test: Retention performance on the object–word association task across immediate, wake, and sleep conditions. *We found a *group* × *condition type* interaction at the 4-h and 24-h delays (see *Results* and *SI Appendix*).

equivalent on basic cognitive scales and background factors (*SI Appendix*, Table S1). Despite equivalent retention at 5 min (Fig. 1*B* and *SI Appendix*, Table S2; $P = 0.39$, alpha Holm–Bonferroni adjusted for six comparisons), groups differed across the wake and nap conditions at 4 h [Fig. 1*C*; significant group × condition interaction, $F(1, 47) = 74.68$, $P < 0.001$, P adjusted = 0.006]. The DS group retained less than the TD group when tested after sleep ($P < 0.001$; $d = 2.57$, P adjusted = 0.006), but were more accurate in the wake condition ($P < 0.001$; $d = -1.28$, P adjusted = 0.006). While the TD group demonstrated a benefit from the nap ($P < 0.001$, P adjusted = 0.006), children with DS were significantly more accurate after wake than following a nap ($P < 0.001$, P adjusted = 0.006), with the same pattern of significance 24 h later (*SI Appendix*, Table S2).

While exploratory analyses showed total nap time and sleep onset latency did not differ across the groups (*SI Appendix*, Table S3), children with DS spent significantly less time in REM sleep ($P = 0.01$, Holm–Bonferroni adjusted for four comparisons, $P = 0.04$), consistent with the past literature. The 44.44% of the group with DS vs. 5.9% of the TD group failed to enter into REM. The percentage of time spent in other sleep stages did not differ between groups (Fig. 2*A*). We examined group differences in EEG power-density spectra in each sleep stage normalized for overall EEG power (relative power). Bootstrap tests (24) found that the DS group had reduced relative power in lower N3 delta, from 2.0 to 3.0 Hz (see Fig. 2*B*).

SI Appendix, Table S4 shows the correlations between sleep architecture and retention in both groups at 4 and 24 h. The groups did differ in the correlation between % REM and retention at 4 h in the nap condition ($z = -2.57$, $P = 0.01$, P adjusted = 0.04), but no other tests of the differences of correlation were significant at 4 h (Fig. 3). In other exploratory analysis, we found that sleep spindle density, wake after sleep onset, and oxygen level (SaO2) did not relate to word recognition after sleep in either group.

Discussion

In this study, we trained children to learn novel words across intervals containing a nap and wake. Using measures of sleep physiology collected in their home environment, we investigated how sleep quality, architecture, and quantitative EEG power during the nap related to retention. First, we found that naps function differently across different populations. Specifically, while naps were beneficial for long-term retention in TD children, they were detrimental in children with DS. This study demonstrates a nap-dependent learning deficit in a subpopulation of participants. This result has implications for the design of treatment protocols that affect learning and memory in children with DS. Second, our findings match other recent work suggesting a role for REM sleep in verbal learning.

Typically developing children benefit from the nap, but they are prone to substantial interference or memory decay during

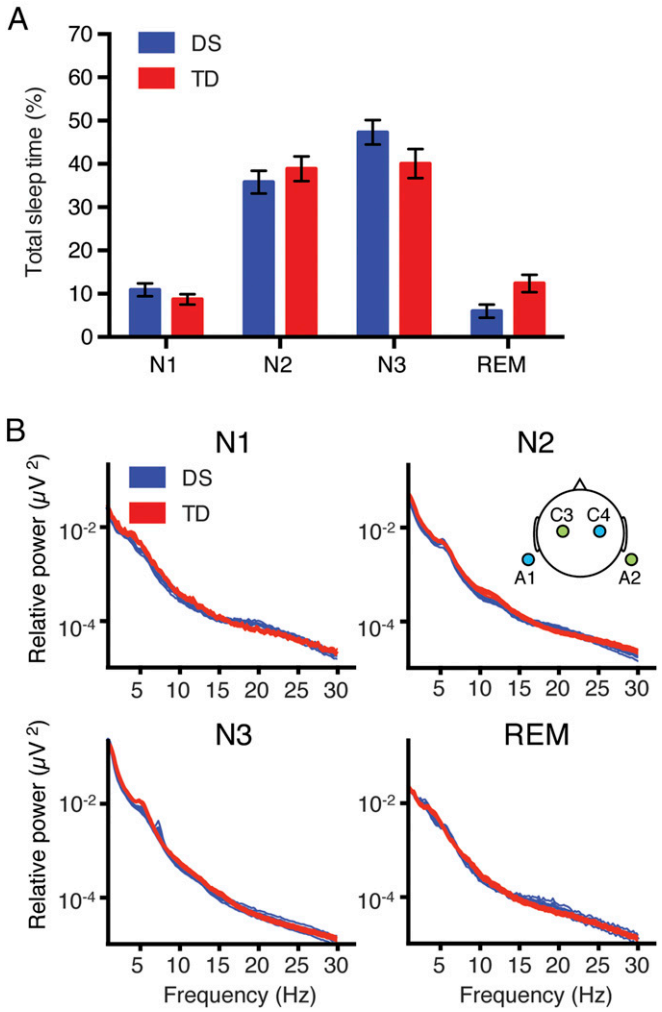

Fig. 2. Sleep architecture and power spectra in children with DS and TD toddlers. (*A*) Sleep architecture in children with DS and TD toddlers. Using a *t* test we showed that children with DS spent significantly less minutes in REM sleep (Holm–Bonferroni adjusted for four comparisons, $P = 0.04$). (*B*) Relative EEG power from 0.2 to 32 Hz at 0.2-Hz resolution for each stage for children with DS (blue line) and the TD controls (red). Bootstrapped tests found that the DS group had reduced relative power in N3 delta, from 2.0 to 3.0 Hz, $P < 0.001$.

periods of wake at both 5-min and 4-h delays. This result adds to a growing literature linking sleep with declarative knowledge acquisition, including word learning (1, 24, 25). In the TD group only, 4-h retention of the object-label association correlated with increased time in REM, consistent with previous studies indicating a role of REM sleep in the consolidation of language-related learning (17, 21, 25). Our results indicate that REM is also important for active consolidation of new arbitrary labels. While REM plays a role in general neural development, it has been more recently implicated in the process of consolidating and integrating new information into existing networks (26). While our method of in-home data collection may have allowed for more natural naps and increased % REM in children, our findings should be replicated in larger samples.

An unexpected finding was that children with DS retained more at 4 and 24 h if they remained awake in the 4 h after training than if they napped, relative to TD children. In other words, memory performance in DS, but not in TD children, decreased if they napped, and this loss was not recovered with nighttime sleep. Furthermore, we observed that DS children expressed a reduction in REM sleep.

What mechanisms could lead to learning loss in DS across a nap? First, sleep has been suggested to contribute to active consolidation as well as synaptic downscaling (8). Given that the group with DS shows nap-dependent memory loss, one candidate mechanism could be excessive synaptic downscaling. However, this explanation seems unlikely, given that the children in the DS group with a higher percentage of N3, the stage considered to be most important for downscaling, showed a non-significant positive correlation to retain more at 4 h than children with lower percentages of N3 (*SI Appendix*, Table S5). An alternative mechanism that could explain our results is that synaptic remodeling must take place within the hippocampus during REM to accurately retain these associations. The hippocampus plays a central role in coordinating system-wide reactivation, and DS is a condition associated with hippocampal impairment and dysfunction. Specifically, mouse models of DS show less input from dentate gyrus, overactive but less discriminant activity in CA3, and poorer place field stability over time (27). Dentate gyrus and CA3 subfields serve pattern separation and completion, and children with DS show deficits in spatial and temporal pattern separation (28). Therefore, one speculation is that the hippocampal circuit replays errant and nonspecific activity patterns during sleep in DS, which, in turn, interferes with memory consolidation, as previously has been suggested might be the outcome of poor hippocampal representations (29). While our data suggest that the learning in the nap was an important factor for long-term retention, more work must examine if the processes operating in naps also extend to nighttime sleep.

In general, more work is needed to understand the impact of reduced REM in naps and nighttime sleep in light of recent findings describing links between REM and learning. Specifically, while our data suggest that REM may be important in naps, other work has suggested that the combination of NREM and sufficient amounts of REM are required to influence learning, with entry into brief intervals of REM actually impairing learning (30). Our data suggest that greater proportions of REM are beneficial in typical children, but the numbers of children who had NREM but no REM sleep are too few to determine any thresholds for learning based on the amount of REM in DS. In total, our current findings suggest a sleep-dependent loss of

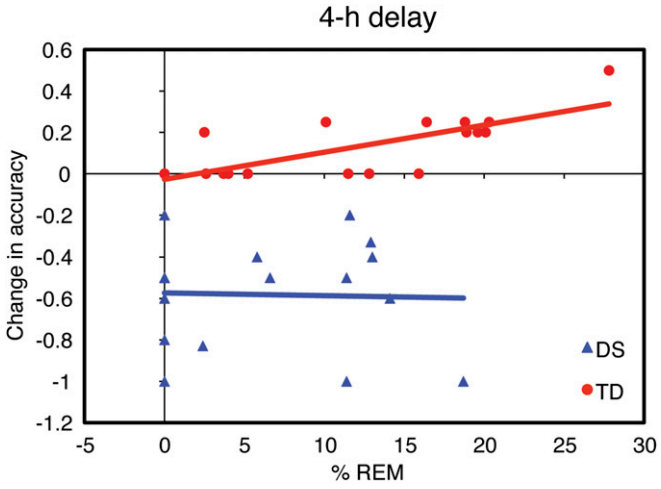

Fig. 3. Associations between retention and sleep variables. Correlations between the percent of time spent in REM sleep and retention after the sleep period at the 4-h delay. Using Fisher's *z* tests of differences in correlations we showed that the groups did differ in the correlation between % REM and retention at 4 h (*P* adjusted = 0.04).

learning in a population with reduced REM sleep in naps, highlighting an important role for REM in young children's memory retention.

These findings lay the groundwork for future investigations of sleep and learning in widely available and well-characterized rodent models of DS. Stemming from basic science with animal models, potential pharmacological interventions for supporting cognitive development in children with DS have emerged. Little attention has been paid to the role of sleep in moderating treatment outcomes in this group. Our data suggest that sleep should be taken seriously as an important source of variability when considering pharmacotherapies and behavioral interventions, and therapeutic approaches to sleep disturbances should be expanded in at-risk pediatric groups such as DS (27, 31).

Our data show that naps benefit knowledge acquisition in young TD children, but not in children with DS. Further, naps should be long enough to allow entry into sufficient REM sleep to be of full benefit. In children with DS, the picture may be different, as our data indicate that naps result in knowledge loss. These findings provide a unique perspective on the underlying mechanisms of this common intellectual disability and suggest that the sleep profile must be carefully considered in future work.

Materials and Methods

Participants. A total of 66 children were contacted through local and parent organizations and advertisement in the Tucson and Phoenix areas, Arizona. A total of 16 children (11 DS, 5 TD, proportion completed vs. noncompleted not significantly different across groups) [X^2 (1, 51) = 1.34, $P = 0.25$] did not end up participating after recruitment for reasons that included (*i*) difficulty engaging in the initial testing session and (*ii*) parent cancelation. Of the 50 children who completed the behavioral protocols across all sessions, 25 were children with DS (13 female; mean age = 54.16 mo, SD = 9.49; range = 41–84 mo). DS (trisomy 21) was verified by karyotype report or medical records. The mean Leiter-3 nonverbal IQ (standard score) was 86.52 ± 11.52 (range = 63–108). Exclusion criteria included the presence of mosaicism and autism spectrum disorder diagnosis. Participants with DS had equivalent nonverbal IQ raw scores to a group of TD children based on the Leiter-3. The control group in this study consisted of 25 TD children (mean age = 33.38 mo, SD = 5.05, range = 26–50 mo) recruited through contact with public parent organizations and advertisement. Children without DS were screened before enrollment to rule out: language delays, neurological conditions, or autism spectrum disorder. We tested 25 TD children, but one child was removed from the analysis due to poor attention during the task session. There were no significant differences between the two groups in total nonverbal summary score on the Leiter-3 [t (47) = −0.93, $P = 0.36$]. Despite age differences resulting from a mental age-matching approach, both groups had equivalent background factors, including gender, body mass index (BMI), ethnicity, and socioeconomic status (*SI Appendix*, Table S1). We included children who habitually napped (4–7 naps per week), given previous findings showing long-term sleep-dependent benefits only in habitual nappers (6).

Procedure. All procedures were approved by the University of Arizona Biomedical Institutional Review Board, and informed consent was obtained from all parents. Participants were tested in their homes in a location with minimal distractions. Each child was tested with an object-label pairing task in three counterbalanced, within-subject conditions separated by 1–2 wk: (*i*) 5-min delay, (*ii*) test after wake, and (*iii*) test after a nap (sleep condition). Every condition included two phases: training and test. During training, children were asked to play a computer game to become an astronaut on a mission to Mars to find new toys. For each condition (5-min delay, wake, and sleep), children were exposed to three novel object-label mappings and three novel unlabeled distractors, resulting in a total of nine target objects and nine distractors. Each object was presented for 11 s paired with either the recording "Look! A dake. Touch the dake. Wow! A dake!" if it was a target object, or with "Hey! Look at that! Touch that. Cool! Look at that" if it was a distractor. Language included "exclamation, touch, exclamation" with the exclamations ("Look!," "Wow!," "Cool!," "Hey, look!") chosen randomly. After presenting the objects, children were tested immediately after the training using the method described for the test phase (see below). To eliminate encoding differences as the source of any consolidation differences, we equated baseline learning in both groups by training each to a set criterion (four out of six correct trials, 66.67%). If children did not reach criterion after one block of training they participated in additional blocks of training.

In the test phase, children were prompted to point to the target object (i.e., "Which one is the dake? Point to the dake!") from among four possibilities: the target-labeled object, a previously labeled object to test if the selection reflected recognition of the specific label–object association, and two distractors to prevent children from choosing based solely on familiarity. Children received six trials of a four-choice forced alternative recognition test presented with quadrant position of each object randomized on each trial. In the 5-min delay condition, children received the recognition test 5 min after the training phase, whereas in the wake and nap conditions, children were tested after 4 h as well as 24 h later to examine long-term retention. While the 5-min delay condition was always completed during the first week, wake and nap were counterbalanced (12 participants with DS and 12 TD toddlers were tested with the "5min-Wake-Nap" order and 13 participants with DS and 12 TD toddlers with the "5min-Nap-Wake" order). For the sleep condition, participants were scheduled about 1 h before the usual naptime, and after the training, a home-based sleep study was performed. In the wake condition, training was administered at a time when the participant did not usually nap to avoid sleep deprivation effects. Mock sensor placements were also attempted. Some participants were scheduled in the morning before the nap and others after the nap to control for time-of-day effects on learning (32).

The time of the training was similar across conditions and groups, ruling out circadian effects. To control for baseline differences on the object-label task, we calculated the adjusted change in recognition at 4- and 24-h delays relative to the baseline performance at training [(delayed − baseline)/baseline] (6, 33). Behavioral ratings of attention were assessed at encoding and at the 24-h delay test with an experimenter-rated scale from 1 to 5 (5 equal to higher levels of attention) to control for potential differences in attention between the wake and the nap delays. The same ratings were also used to measure sleepiness before the encoding phase and at the 4-h delay in a subsample of children (DS = 10 and TD = 6). This measure was introduced to control for confounding factors linked to the nap period that might influence performance on the task (e.g., sleep inertia, reduced fatigue).

Stimuli. Pictures of novel objects, chosen to be relatively different from one another, were matched with nonword labels, in which neighborhood size (34) and biphone frequency were controlled (35). Children were tested on consonant–vowel–consonant (CVC) labels, the most representative of the structure of English. The following labels were used for set 1: dake, tobe, peen; set 2: wame, bope, neek; and set 3: tade, doke, meep. Two versions of the task were used in this study, such that distractors in version 1 were used as target objects in version 2, and vice versa. As a result, labels were associated with different target objects in the two versions to control for some objects being more memorable than others. Ten participants with DS and 13 TD toddlers were randomly assigned to version 1, whereas 15 participants with DS and 11 TD toddlers received version 2. Administration order was randomized across conditions to avoid pairing one condition with the same stimuli version across participants (e.g., version 1 was paired with the nap condition for one child and with the wake condition for another child).

Assessment of Nap Physiology.

Home-based polysomnography. All children underwent polysomnography (PSG) in their homes with the experimenter attending to maximize participant compliance (Compumedics Somte PSG system Compumedics USA, Inc.). We conducted our nap assessments in-home in both TD children and children with DS, allowing for as natural an examination of naps and learning as possible. Very few studies have examined the underlying mechanisms of nap-dependent learning in young children, and often the sleep physiology has been conducted in a laboratory setting, which may limit the acquisition of natural naps containing REM (36). The feasibility of ambulatory PSG has been extensively documented in special populations such as individuals with DS (37, 38) and children with autism spectrum disorder (39) as well as in typically developing young children as early as 2 y (40, 41). Ambulatory PSG is by nature less intrusive for children and their families, and sleep quality is better compared with laboratory polysomnography (42, 43).

PSG was conducted during the nap in 18 children with DS and 17 TD toddlers. Mock PSG, including the placement of a few electrodes and exposure to the leads, was conducted during the wake interval. In accordance with the International 10–20 system (44), our PSG recording included EEG (sampled at 250 Hz) at central derivations (C3/A2, C4/A1), two electrooculogram channels (EOG), and two electromyogram channels (EMG). The presence of sleep disturbances was evaluated with thoracic and abdominal

displacement (inductive phlethysmography bands) and a finger pulse oximeter (Nonin 8000J Series Flex SpO2 Sensor; Nonin Medical, Inc.). Pulse oximetry alone has been used to assess sleep-disordered breathing as one alternative of PSG in young children (45), and a 3% oxygen desaturation index may be used as an estimate of the severity of OSA (46). Sleep was visually scored by a registered polysomnographic technologist according to the American Academy of Sleep Medicine standard criteria, taking into account specific recommendations for pediatric sleep (40). Sleep spindles were visually identified on the C3 and C4 channels by a registered sleep technologist blind to the group membership (TD vs. DS) according to the following criteria: bursts of 12–15 Hz EEG that lasted at least 0.5 s.

Power spectral analyses were based on the central EEG lead C4 because more children retained C4 across the nap. Files were coded for artifact using visual inspection: epochs including bad EEG channels, arousals, or excessive movement were excluded. A Fast Fourier transform was applied using the spectrogram function in Matlab, with a 5-s hamming window to provide power spectral densities (in μV^2). We used a data-driven bootstrapping approach (independent samples in SPSS; 5,000 samples with a 95% confidence interval) to examine group differences in EEG power-density spectra across the entire frequency range (0.6–32 Hz) normalized for overall EEG power (relative power) in each sleep stage. Individual 0.2-Hz bands were examined across this range. Only significant bands surrounded by two other significant bands were included and were considered significant at $P < 0.001$. In previous uses of this technique, multiple comparisons were considered to be controlled because of focus on concurrently significant bands, but here we also report only findings meeting a significance threshold of $P < 0.001$ (47). We also examined the relation between learning and power using a priori defined bands of interest in the delta band in N3 (commonly referred to as slow wave activity, SWA; 1–4.5 Hz).

Actigraphy. Children wore the Actiwatch 2 (Actiwatch 2; Phillips Respironics Mini-Mitter) for at least five consecutive nights on their nondominant wrist (one TD participant was excluded due to not reaching the five-night actigraphy minimum), and parents completed a 1-wk sleep log. Parents were instructed to document all periods of the participant's sleep, including naps and overnight sleep, sleep location, and periods of time when the watch was taken off. The completed sleep log was then used to supplement the analysis of the actigraphy data. Light and activity data were collected in 30-s epochs and analyzed using the Philips Actiware 6.0.2 software packaging (Respironics Actiware 6.0.2). Data were scored using a medium sensitivity (40 activity cpm), with sleep onset and sleep end marked by a period of 3 and 5 min of immobility or more, respectively (48). Epochs detected to have activity counts greater than the medium sensitivity threshold were considered periods of wake, and those below the threshold were considered periods of sleep. Actigraphy data from all overnight sleep and naps in the sleep condition were analyzed for each subject. Variables of interest were sleep efficiency (SE), sleep duration, sleep fragmentation index (FI; measured as the percentage of sleep considered to be restless due to consistent physical movement), and wake after sleep onset (WASO; the number of minutes awake during a sleep period).

Descriptive Measures. After completing the 24-h test delay of the object-label pairing task, participants were assessed on the Leiter International Performance Scale, Third Edition (49). Due to its nonverbal administration, this assessment is often used to assess fluid intelligence in individuals with speech or hearing impairments as well as cognitive delays and has been previously validated in DS (50). To obtain a nonverbal IQ score we administered five cognitive scales including sequential order, form completion, classification and analogies, figure–ground, and matching/repeated patterns. The outcome measure employed in this study was the sum of the five subtests, which was used to match children with DS and the TD group.

Statistical Analyses. All statistical analyses were performed with SPSS 24.0 (IBM Corp.). The distributional properties of each measure (e.g., normality) were examined. We tested if groups were homogenous for descriptive variables such as gender, IQ, and socioeconomic status using χ^2 tests for dichotomous outcomes, *t* tests for continuous and normally distributed outcomes, and the Mann–Whitney *U* test for nonparametric outcomes. To test if groups performed similarly at baseline, we performed a repeated measures ANOVA for both wake and nap conditions. We then examined group differences in the delayed recognition by performing a 2×2 ANOVA with condition type (wake-delay vs. nap-delay) as the repeated factor and group (DS vs. TD) as the between-subjects factor. These analyses were conducted at both 4- and 24-h delays. If an interaction was found, individual group differences in the wake and nap conditions were analyzed using planned comparisons based on hypotheses generated from past work (*t* tests; Holm–Bonferroni correction was used for multiple comparisons and was adjusted for the six behavioral tests).

To identify sleep-related factors that might be driving these effects, we first conducted group comparisons on sleep outcome variables derived from polysomnography recorded during the nap by using a *t* test for normally distributed outcomes and a Mann–Whitney *U* test for nonnormal outcomes. Based on expectations of sleep-stage differences from our previous work (51), *P* values were Holm–Bonferroni-corrected for four comparisons. Group differences in the EEG power-density spectra were examined using bootstrapped independent-sample *t* tests, which were considered significant at $P < 0.001$ (47). Associations between relevant sleep physiology variables and retention of the object–word association at 4 and 24 h were examined with Pearson's correlations. Fisher's *z* tests of differences in correlations were conducted to determine if relations between sleep parameters (% REM and N3 delta) and learning differed in DS and TD. These comparisons were Holm–Bonferroni-corrected for a family of four tests. Finally, to determine the relation between sleep disruption and learning in the DS population, we compared children with DS with differences in sleep quality [DS poor sleep (PS) and DS good sleep (GS)] using analyses of variance (ANOVAs). In the supplemental analyses, we evaluate effects with familywise Bonferroni correction per section given the exploratory nature of those additional analyses. For instance, given the importance of examining sleep across a number of parameters in additional exploratory tests, we applied Holm–Bonferroni's *P* value correction to seven group comparisons (i.e., sleep onset latency, sleep efficiency, total sleep time, arousal index, average SaO2 desaturation, WASO, and N2 sleep spindle density; *SI Appendix*, Table S3).

ACKNOWLEDGMENTS. This work would not be possible without the support of individuals with Down syndrome and their families in Arizona. We would also like to thank the late Dr. Michael Harpold for his devotion to the field of Down syndrome. This research was supported by the LuMind Research Down Syndrome Foundation, the Jérôme Lejeune Foundation, and the Molly Lawson Foundation.

1. Henderson L, Weighall A, Gaskell G (2013) Learning new vocabulary during childhood: Effects of semantic training on lexical consolidation and integration. *J Exp Child Psychol* 116:572–592.
2. Tamminen J, Payne JD, Stickgold R, Wamsley EJ, Gaskell MG (2010) Sleep spindle activity is associated with the integration of new memories and existing knowledge. *J Neurosci* 30:14356–14360.
3. Mednick S, Nakayama K, Stickgold R (2003) Sleep-dependent learning: A nap is as good as a night. *Nat Neurosci* 6:697–698.
4. Gómez RL, Bootzin RR, Nadel L (2006) Naps promote abstraction in language-learning infants. *Psychol Sci* 17:670–674.
5. Seehagen S, Konrad C, Herbert JS, Schneider S (2015) Timely sleep facilitates declarative memory consolidation in infants. *Proc Natl Acad Sci USA* 112:1625–1629.
6. Kurdziel L, Duclos K, Spencer RMC (2013) Sleep spindles in midday naps enhance learning in preschool children. *Proc Natl Acad Sci USA* 110:17267–17272.
7. Diekelmann S, Born J (2010) The memory function of sleep. *Nat Rev Neurosci* 11:114–126.
8. Tononi G, Cirelli C (2014) Sleep and the price of plasticity: From synaptic and cellular homeostasis to memory consolidation and integration. *Neuron* 81:12–34.
9. Ashworth A, Hill CM, Karmiloff-Smith A, Dimitriou D (2017) A cross-syndrome study of the differential effects of sleep on declarative memory consolidation in children with neurodevelopmental disorders. *Dev Sci* 20:e12383.
10. Gómez RL, Edgin JO (2015) Sleep as a window into early neural development: Shifts in sleep-dependent learning effects across early childhood. *Child Dev Perspect* 9:183–189.
11. Prehn-Kristensen A, et al. (2011) Reduced sleep-associated consolidation of declarative memory in attention-deficit/hyperactivity disorder. *Sleep Med* 12:672–679.
12. Parker SE, et al.; National Birth Defects Prevention Network (2010) Updated National Birth Prevalence estimates for selected birth defects in the United States, 2004-2006. *Birth Defects Res A Clin Mol Teratol* 88:1008–1016.
13. Martin GE, Losh M, Estigarribia B, Sideris J, Roberts J (2013) Longitudinal profiles of expressive vocabulary, syntax and pragmatic language in boys with fragile X syndrome or Down syndrome. *Int J Lang Commun Disord* 48:432–443.
14. Nadel L (2003) Down's syndrome: A genetic disorder in biobehavioral perspective. *Genes Brain Behav* 2:156–166.
15. Edgin JO (2013) Cognition in Down syndrome: A developmental cognitive neuroscience perspective. *Wiley Interdiscip Rev Cogn Sci* 4:307–317.
16. Boyce R, Williams S, Adamantidis A (2017) REM sleep and memory. *Curr Opin Neurobiol* 44:167–177.
17. Casey SJ, et al. (2016) Slow wave and REM sleep deprivation effects on explicit and implicit memory during sleep. *Neuropsychology* 30:931–945.
18. Poe GR (2017) Sleep is for forgetting. *J Neurosci* 37:464–473.
19. Rasch B, Büchel C, Gais S, Born J (2007) Odor cues during slow-wave sleep prompt declarative memory consolidation. *Science* 315:1426–1429.

20. Antony JW, Gobel EW, O'Hare JK, Reber PJ, Paller KA (2012) Cued memory reactivation during sleep influences skill learning. *Nat Neurosci* 15:1114–1116.
21. Sterpenich V, et al. (2014) Memory reactivation during rapid eye movement sleep promotes its generalization and integration in cortical stores. *Sleep (Basel)* 37: 1061–1075, 1075A–1075B.
22. Ackermann S, Rasch B (2014) Differential effects of non-REM and REM sleep on memory consolidation? *Curr Neurol Neurosci Rep* 14:430.
23. Miano S, et al. (2008) Sleep phenotypes of intellectual disability: A polysomnographic evaluation in subjects with Down syndrome and fragile-X syndrome. *Clin Neurophysiol* 119:1242–1247.
24. Sandoval M, Leclerc JA, Gómez RL (2017) Words to sleep on: Naps facilitate verb generalization in habitually and nonhabitually napping preschoolers. *Child Dev* 88: 1615–1628.
25. Tamminen J, Lambon Ralph MA, Lewis PA (2017) Targeted memory reactivation of newly learned words during sleep triggers REM-mediated integration of new memories and existing knowledge. *Neurobiol Learn Mem* 137:77–82.
26. Li W, Ma L, Yang G, Gan W-B (2017) REM sleep selectively prunes and maintains new synapses in development and learning. *Nat Neurosci* 20:1–16.
27. Fernandez F, Edgin JO (2016) Pharmacotherapy in Down's syndrome: Which way forward? *Lancet Neurol* 15:776–777.
28. Clark CAC, Fernandez F, Sakhon S, Spanò G, Edgin JO (2017) The medial temporal memory system in Down syndrome: Translating animal models of hippocampal compromise. *Hippocampus* 27:683–691.
29. Buzsáki G (1998) Memory consolidation during sleep: A neurophysiological perspective. *J Sleep Res* 7:17–23.
30. Schapiro AC, et al. (2017) Sleep benefits memory for semantic category structure while preserving exemplar-specific information. *Sci Rep* 7:14869.
31. Colas D, Chuluun B, Garner CC, Heller HC (2017) Short-term treatment with flumazenil restores long-term object memory in a mouse model of Down syndrome. *Neurobiol Learn Mem* 140:11–16.
32. Folkard S, Monk TH, Bradbury R, Rosenthall J (1977) Time of day effects in school children's immediate and delayed recall of meaningful material. *Br J Psychol* 68: 45–50.
33. Wilhelm I, Metzkow-Mészàros M, Knapp S, Born J (2012) Sleep-dependent consolidation of procedural motor memories in children and adults: The pre-sleep level of performance matters. *Dev Sci* 15:506–515.
34. Balota DA, et al. (2007) The English Lexicon Project. *Behav Res Methods* 39:445–459.
35. Vitevitch MS, Luce PA (2004) A web-based interface to calculate phonotactic probability for words and nonwords in English. *Behav Res Methods Instrum Comput* 36: 481–487.
36. Kurth S, et al. (2016) Development of nap neurophysiology: Preliminary insights into sleep regulation in early childhood. *J Sleep Res* 25:646–654.
37. Breslin J, et al. (2014) Obstructive sleep apnea syndrome and cognition in Down syndrome. *Dev Med Child Neurol* 56:657–664.
38. Brockmann PE, et al. (2016) Sleep-disordered breathing in children with Down syndrome: Usefulness of home polysomnography. *Int J Pediatr Otorhinolaryngol* 83: 47–50.
39. Maski K, et al. (2015) Sleep dependent memory consolidation in children with autism spectrum disorder. *Sleep (Basel)* 38:1955–1963.
40. Grigg-Damberger M, et al. (2007) The visual scoring of sleep and arousal in infants and children. *J Clin Sleep Med* 3:201–240.
41. Kurth S, Achermann P, Rusterholz T, Lebourgeois MK (2013) Development of brain EEG connectivity across early childhood: Does sleep play a role? *Brain Sci* 3:1445–1460.
42. Bruyneel M, Ninane V (2014) Unattended home-based polysomnography for sleep disordered breathing: Current concepts and perspectives. *Sleep Med Rev* 18:341–347.
43. Marcus CL, et al. (2014) Feasibility of comprehensive, unattended ambulatory polysomnography in school-aged children. *J Clin Sleep Med* 10:913–918.
44. Homan RW, Herman J, Purdy P (1987) Cerebral location of international 10-20 system electrode placement. *Electroencephalogr Clin Neurophysiol* 66:376–382.
45. Kaditis AG, et al. (2016) Obstructive sleep disordered breathing in 2- to 18-year-old children: Diagnosis and management. *Eur Respir J* 47:69–94.
46. Oeverland B, Skatvedt O, Kvaerner KJ, Akre H (2002) Pulseoximetry: Sufficient to diagnose severe sleep apnea. *Sleep Med* 3:133–138.
47. Saletin JM, Coon WG, Carskadon MA (2017) Stage 2 sleep EEG sigma activity and motor learning in childhood ADHD: A pilot study. *J Clin Child Adolesc Psychol* 46: 188–197.
48. Meltzer LJ, Montgomery-Downs HE, Insana SP, Walsh CM (2012) Use of actigraphy for assessment in pediatric sleep research. *Sleep Med Rev* 16:463–475.
49. Roid GH, Miller LJ, Pomplun M, Koch C (2013) *Leiter International Performance Scale, (Leiter-3)* (Western Psychological Services, Los Angeles).
50. Liogier d'Ardhuy X, et al. (2015) Assessment of cognitive scales to examine memory, executive function and language in individuals with Down syndrome: Implications of a 6-month observational study. *Front Behav Neurosci* 9:300.
51. Edgin JO, et al. (2015) Sleep disturbance and expressive language development in preschool-age children with Down syndrome. *Child Dev* 86:1984–1998.